International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Comparison of Antimicrobial Activity of Extracted Gallotannin from Rheum Palmatum, Syzygium Aromaticum and Terminalia Chebula Against Methicillin-Resistant Staphylococcus Aureus (MRSA)

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ABSTRACT

Comparison of antimicrobial activity of extracted gallotannin from *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* against Methicillin-resistant *Staphylococcus aureus* (MRSA). The objective is to compare the effectiveness of antimicrobial activity against MRSA, which is an antimicrobial resistance, using gallotannin extracts from *Rheum palmatum, Syzygium aromaticum, and Terminalia chebula.*The study also aims to investigate how MRSA became ineffective to antibiotic. *Rheum palmatum, Syzygium aromaticum*, and *Terminalia chebula* were extracted using ethanol at different concentration, and the functional groups of the extracts were examined using FTIR micro-spectroscopy, which proved that gallotannin was found in each extract. The effectiveness of the herb extracts against MRSA was tested using the Disc Diffusion Test. Each position contained different substances: Point A contained Oxacillin and Ampicillin, Point B contained the herbal extracts, Point C contained Control Ethanol 95% and Point D contained Oxacillin and Ampicillin with the herbal extracts. According to the experiment result, the herbal extracts were more efficient than both antibiotic and antibiotic combined with extracts in resisting MRSA. Among the extracts, the biggest inhibitor zone was created around the extract of *Syzygium aromaticum* with 95% ethanol. This suggests that the extract from *Syzygium aromaticum* is the most effective antimicrobial against MRSA.

Keywords: MRSA, Gallotannin, *Rheum palmatum, Syzygium aromaticum, Terminalia chebula*

1. Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of *Staphylococcus aureus* that is resistant to β-lactam antibiotics, such as Penicillin, Oxacillin, and Methicillin. MRSA can enter the body through wounds and the bloodstream, causing illnesses such as endocarditis. When the infection occurs on the skin, it can cause pustules, abscesses, or skin infections. In certain cases, the inability to save the patient's life may arise from the effects of MRSA, which makes it harder or impossible to treat. According to data gathered between 2017 and 2018 and 2020 and 2021, Genomics of Hospital, Community and Livestock Associated Methicillin-Resistant *Staphylococcus aureus* for Applications in Medicine and Public Health revealed the majority of patients with MRSA infections are male with the average age of 58.52±19.43

years. After MRSA was found in phlegm, 75.9% of patients experienced complications that caused skin and tissue infections. The data shows that the trend of MRSA patients is rising significantly, and this should be frequently monitored.

Many studies have found that *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* contain gallotannins, which are compounds in the tannin group. Gallotannin has the property of precipitating *Staphylococcus aureus* proteins that also inhibit and interfere the activity of β-lactamase, an MRSA enzyme that breaks down the β-lactam ring in β-lactam antibiotics that cause antimicrobial resistance. Therefore, we are interested to use gallotannin from *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* extracts by testing the extracts for their effectiveness of antimicrobial activity against MRSA. By working with these herbs, we aim to use the potential of this study to develop and create alternative treatments for the antimicrobial resistance by using these herbs.

So, the objectives of this research are:

- To examine the presence of gallotannins in the extracts of *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula*
- To compare antimicrobial activity of extracted gallotannin from *Rheum palmatum, Syzygium aromaticum* and *Terminalia chebula* against Methicillin-resistant *Staphylococcus aureus (MRSA)*

2. Method and Experimental Details

Part 1: Extraction of gallotannin from *Rheum palmatum*, *Syzygium aromaticum*, and *Terminalia chebula*, and Examination of gallotannin Properties

- **Extraction of gallotannin from the Herbs:** Measuring 10 grams of each herb with 100 milliliters of solvent, using two sets of solvents with different concentrations (30% ethanol and 95% ethanol). The substance was heated at 60°C for 2 hours. In addition to preventing ethanol evaporation during the extraction process, the extraction with 95% ethanol solvent was covered with aluminum foil (with holes). The herbal extract was filtered using a Büchner funnel after the extraction. Pipette 20 milliliters of the herbal extract then placed it in the Water Bath to evaporate the ethanol and water.
- **Examination of gallotannin Properties Using FT- IR Spectroscopy:** The study found that gallotannins contain these main bonds: ether, ester, and phenol and the frequency ranges for these bonds are 1,000-1,300, 1,700, and 3,200-3,500 cm^{-1}, respectively. These data were used to compare with the spectrograph of gallotannin extracts from *Rheum palmatum*, *Syzygium aromaticum*, and *Terminalia chebula* to verify gallotannin property.

Part 2: Testing the effectiveness of antimicrobial activity of extracted gallotannin from *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* against Methicillin- resistant *Staphylococcus aureus* (MRSA)

- **Preparation of gallotannin Extracts and Discs:** gallotannin extracts from Part 1 were adjusted for concentration by mixing with 95% ethanol in a 1:4 Ratio. A vortex mixer and an ultrasonic cleaner were used to obtain the extracts in liquid form. Pipette 20 microliters of the extracts and dropped them onto blank discs and antibiotic discs (Oxacillin, Ampicillin).
- **Preparation of Bacterial Culture Media:**

1. **Liquid Culture Medium Preparation:** 20 milliliters of water mixed with 0.26 grams of Lysogeny Broth (LB) were prepared and divided into 10 milliliters per test tube.

2. **Solid Culture Medium Preparation:** 15.2 grams of Mueller Hinton Agar (MHA) and 400 milliliters of water were mixed in the microwave for one to two minutes. Continue doing this until the medium is clear without sediment.
3. **Sterilization of Equipment:** All equipment was sterilized in an autoclave at 121.5°C and 15 psi.
4. **Plating of Sterile MHA:** 400 milliliters of sterile MHA were poured into petri dishes using a peristaltic pump, with each dish containing 20 milliliters. The medium was left to solidify.

- **Testing the effectiveness of antimicrobial activity of extracted gallotannin using Disc Diffusion Method:** MRSA was cultured in 10 milliliters of LB at 37°C for 24 hours in a shaking incubator. The concentration of bacteria was adjusted in 0.85% NaCl, compared to the 0.5 McFarland standard. A cotton swab was used to spread the bacteria on the MHA surface. Discs were placed in each petri dish as following point:
  - Point A: Antibiotic (Oxacillin, Ampicillin)
  - Point B: gallotannin Extract
  - Point C: 95% Ethanol (Control)
  - Point D: Antibiotic with gallotannin Extract

Table 1: Position of the discs

Petri dish	Point A	Point B	Point C	Point D
1	Oxacillin	*Rheum palmatum* extract (30% Ethanol)	Ethanol 95%	Oxacillin + *Rheum palmatum* extract (30% Ethanol)
2	Oxacillin	*Syzygium aromaticum* extract (30% Ethanol)	Ethanol 95%	Oxacillin + *Syzygium aromaticum* extract (30% Ethanol)
3	Oxacillin	*Terminalia chebula* extract (30% Ethanol)	Ethanol 95%	Oxacillin + *Terminalia chebula* extract (30% Ethanol)
.4	Oxacillin	*Rheum palmatum* extract (95% Ethanol)	Ethanol 95%	Oxacillin + *Rheum palmatum* extract (95% Ethanol)
5	Oxacillin	*Syzygium aromaticum* extract (95% Ethanol)	Ethanol 95%	Oxacillin + *Syzygium aromaticum* extract (95% Ethanol)
6	Oxacillin	*Terminalia chebula* extract (95% Ethanol)	Ethanol 95%	Oxacillin + *Terminalia chebula* extract (95% Ethanol)
7	Ampicillin	*Rheum palmatum* extract (30% Ethanol)	Ethanol 95%	Ampicillin + *Rheum palmatum* extract (30% Ethanol)
8	Ampicillin	*Syzygium aromaticum* extract (30% Ethanol)	Ethanol 95%	Ampicillin + *Syzygium aromaticum* extract (30% Ethanol)

9	Ampicillin	*Terminalia chebula* extract (30% Ethanol)	Ethanol 95%	Ampicillin + *Terminalia chebula* extract (30% Ethanol)
10	Ampicillin	*Rheum palmatum* extract (95% Ethanol)	Ethanol 95%	Ampicillin + *Rheum palmatum* extract (95% Ethanol)
11	Ampicillin	*Syzygium aromaticum* extract (95% Ethanol)	Ethanol 95%	Ampicillin + *Syzygium aromaticum* extract (95% Ethanol)
12	Ampicillin	*Terminalia chebula* extract (95% Ethanol)	Ethanol 95%	Ampicillin + *Terminalia chebula* extract (95% Ethanol)

The effectiveness was observed by measuring the inhibition zone with a ruler after incubating for 24 hours at 37°C. The results were recorded in a data table. The tests were conducted with two types of drugs and gallotannin extracts from herbs extracted with different solvent concentrations, resulting in 12 petri dishes with different points for disc placement.

3. Experimental Results and Discussion

3.1 Experimental Results

Part 1: Extraction of gallotannin from *Rheum palmatum, Syzygium aromaticum,* and *Terminalia chebula*, and Examination of gallotannin Properties

- **Extraction of Gallotannin from the Herbs**

In the extraction process, we used a 1:10 ratio for herbs and ethanol solvent and set it to 60 degrees celsius for 2 hours. Then filtering the extracts from three herbs and two solvents using a Büchner funnel. Upon examination of the filtered extracts, it was found that the 30% ethanol *Rheum palmatum* extract had fine particles that had absorbed plenty of solvent and was difficult to pass through the filter. The extract of *Syzygium aromaticum*, and *Terminalia chebula* passed through the filter easily and when set alone, there will be sediment at the bottom. On the other hand, the extracts of the three herbs, using 95% ethanol as the solvent, easily pass through filter paper and the extracts showed different colors.

- **Examination of Gallotannin Properties Using FT-IR Spectroscopy**

From the analysis of the graphs, it can be observed that all spectrograms of the extracts from the three herbs show a area in the frequency range of 3000-3500 cm^{-1}, which indicates the presence of phenolic bonds. Additionally, there is a peak around 1700 cm^{-1}, representing ester bonds, and ether bonds in the frequency range of 1000-1300 cm^{-1}, visible in all six graphs of the gallotannin extracts from *Rheum palmatum, Syzygium aromaticum, and Terminalia chebula.* Therefore, it can be concluded that the extracts from these three herbs contain bonds similar to those in gallotannins, and thus, *Rheum palmatum, Syzygium aromaticum,* and *Terminalia chebula* extracts have gallotannin properties.

Part 2: Testing the effectiveness of antimicrobial activity of extracted gallotannin from *Rheum palmatum, Syzygium aromaticum* and *Terminalia chebula* against Methicillin- resistant *Staphylococcus aureus* (MRSA)

In the experiment, disc paper of antibiotic, gallotannin extracts, 95% ethanol (control), and antibiotic with gallotannin extracts were placed on points A, B, C, and D of the agar plates, respectively. The plates were then incubated in an incubator for 24 hours at 37°C. After the incubation period, the agar plates showed the inhibitor zones around the disc at the different points were measured and recorded in the results table

as follows:

Table2: Diameter of inhibitor zones around the discs

Petri dish	Point A (mm.)	Point B (mm.)	Point C (mm.)	Point D (mm.)
1	0	16.0	0	19.0
2	0	23.0	0	21.0
3	0	18.5	0	17.0
4	0	20.5	0	18.0
5	0	24.0	0	22.0
6	0	18.0	0	18.0
7	10.0*	16.5	0	16.0
8	9.0*	22.5	0	21.0
9	10.0	15.0	0	16.0
10	11.0*	20.0	0	19.0
11	9.5	22.0	0	21.5
12	10.0*	16.0	0	17.0

* There is MRSA in inhibition zone

From the results table, it can be seen that no inhibitor zone appeared around the Oxacillin disc, whereas a small inhibitor zone was observed around the Ampicillin disc. This indicates that MRSA is more resistant to Oxacillin than to Ampicillin, as Oxacillin is ineffective against MRSA. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the minimum inhibitory zone diameter for Oxacillin that qualifies as effective against MRSA is 18 mm, while for Ampicillin it is 29 mm.

Observing point A and point D on plates 1-6, it was found that no inhibitor zone appeared around the Oxacillin disc, while inhibitor zones were observed around the disc of antibiotic and gallotannin extracts. The inhibitor zones measured from 18 mm or more in diameter, indicating that gallotannin extracts from all three herbs enhanced the effectiveness of Oxacillin against MRSA. Among the extracts, the one that best improved the effectiveness of Oxacillin was the gallotannin extract from *Syzygium aromaticum*, extracted with 95% ethanol. This is evident from the greatest inhibitor zone, measuring 22 mm, around the Oxacillin disc with *Syzygium aromaticum* extract.

On plates 7-12, it was observed that the Ampicillin disc (point A) produced a small inhibitor zone ranging from 9 to 11 mm in diameter. On the other hand, the Ampicillin disc with gallotannin extract (point D) showed a larger clear zone, ranging from 16 to 21.5 mm. Although this does not meet the Clinical and Laboratory Standards Institute (CLSI) criteria for effective MRSA resistance, the gallotannin extract does enhance the effectiveness of Ampicillin, as indicated by the larger inhibitor zone compared to point A.

3.2 Discussion

Gallotannin is a substance that inhibits bacteria. This project studied the effectiveness of gallotannin extracted from *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* against MRSA (Methicillin- resistant *Staphylococcus aureus*) by comparing its effectiveness with Oxacillin and Ampicillin. It was found that there was no inhibition zone around the Oxacillin and Ampicillin discs, indicating the absence of bacterial inhibition. The *Syzygium aromaticum* extract with a 95% concentration

produced the greatest inhibition zone (24.0 mm in diameter), followed by *Syzygium aromaticum* extract with 30% ethanol concentration (23.0 mm in diameter), and *Terminalia chebula* extract with 95% ethanol concentration (20.5 mm in diameter).

When the extracts were combined with Oxacillin, the *Syzygium aromaticum* extract with 95% ethanol produced the greatest inhibition zone, measuring 22 mm in diameter. According to the Clinical and Laboratory Standard Institute (CLSI) guidelines, an inhibition zone diameter of 18 mm or more is required for Oxacillin to be considered effective against MRSA. However, when the extracts were combined with Ampicillin, the *Syzygium aromaticum* extract with 95% concentration produced the greatest inhibition zone of 21.5 mm in diameter. This did not meet the CLSI guideline, which specifies that an inhibition zone diameter of 29 mm or more is necessary for Ampicillin to be considered effective against MRSA.

4. Conclusion

This project, Comparison of antimicrobial activity of extracted gallotannin from *Rheum palmatum*, *Syzygium aromaticum* and *Terminalia chebula* against Methicillin- resistant *Staphylococcus aureus* (MRSA), found that gallotannins are present in all three herbs extracts. When testing the effectiveness of these extracts against MRSA, the *Syzygium aromaticum* extract with 95% ethanol produced the greatest inhibition zone, followed by the *Syzygium aromaticum* extract with 30% ethanol. In comparison, Oxacillin showed an inhibition zone of 0.0 mm, and Ampicillin had an average inhibition zone of 0.95 mm. However, the herb extracts had more than 16.0 mm of inhibition zone. Therefore, it can be concluded that gallotannin extracts from these herbs are more effective against MRSA than Oxacillin and Ampicillin.

When comparing the effectiveness of the extracts by observing the inhibition zones around the antibiotic discs and the discs with gallotannin extracts, we found that the extracts enhanced the anti-drug resistance. Oxacillin with *Syzygium aromaticum* extracted by 95% ethanol, produced an inhibition zone with a diameter of 22.0 mm. This meets the standard for Oxacillin, which requires an inhibition zone diameter of 18 mm or more to be considered effective against MRSA. For Ampicillin, the gallotannin extract increased the inhibition zone size, but it did not reach the standard requirement of 29 mm. Therefore, it can be concluded that gallotannin extracts enhance the effectiveness of againsting antibiotic resistance.

5. References

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